REMARKS

Claims 1, 11-13, and 23-25, 35, and 36 have been amended, claims 53-61 have been added, and claims 2, 4, 6-10, 14, 16, 18-22, 26, 28, and 30-34 have been canceled. Claims 3, 5, 15, 17, 27, 29, and 37-52 were previously canceled. Upon entry of this amendment, claims 1, 11-13, 23-25, 35, 36, and 53-61 will be pending.

More specifically, claim 1 has been amended to recite a bone condition associated with breakdown of bone tissue or bone loss. Support for this amendment can be found, e.g., at paragraph [0006] of the specification (paragraph numbers refer to the specification as published under U.S. Patent Publication No. 2006/0116318). Claims 1, 13, and 25 have been amended to specify an effective amount of a peptide comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 1, 2, or 3. Support for this amendment can be found, e.g., in original claim 7 and at paragraph [0025] of the specification. Claim 25 has also been amended to replace "modulating" with "inhibiting" to promote clarity. Support for this amendment can be found, e.g., at paragraph [0058] in the specification. Support for amended claims 11, 12, 23, 24, 35, and 36 can be found, e.g., at paragraph [0026]. Support for new claims 53-61 can be found, e.g., in original claims 2 and 3 and at paragraph [0025]. No new matter is added.

The claim amendments made herein have been made solely to expedite prosecution of the instant application and should not be construed as an acquiescence to any of the Examiner's rejections.

The following remarks are in response to the Office Action mailed March 20, 2007 ("the Office Action") and the Advisory Action mailed October 3, 2007 ("the Advisory Action").

35 U.S.C. § 112, First Paragraph

Written Description

The Office rejected claims 1, 2, 4, 6-14, 16, 18-26, 28, and 30-36 under 35 U.S.C § 112, first paragraph, for allegedly failing to meet the written description requirement.

First, applicants note that the Office Action and the Advisory Action acknowledge adequate written description for "the species of SEQ ID NOs:1, 2, and 3, peptide fragments

comprising residues 17-34 of these sequences and peptides having at least 95% homology with SEQ ID NOs: 1, 2, and 3" (see the Advisory Action at page 2). The claims, as amended, require peptides comprising an amino acid sequence at least 90% identical to SEQ ID NOs:1, 2, or 3. Thus the claims, as amended, include peptides that fall within the scope of "peptide fragments comprising residues 17-34" of the preptin sequences recited in the specification. Regarding the 90% identity limitation in the amended claims, applicants note that SEQ ID NOs:1, 2, and 3, each have 34 amino acids. Ninety percent identity to a 34 amino acid peptide requires identity to **at least 30** of the amino acid residues in the sequence. Ninety-five percent identity requires identity to **at least 32** of the amino acid residues, a difference of **only two residues** as compared to 90%. Accordingly, the genus of peptides requiring 90% identity is not excessively broader than the genus requiring 95% identity. The genus requiring 90% identity is supported by the disclosure of the specification (see, e.g., paragraphs [0007]-[0020]). It is believed that the amendments overcome this rejection. In addition, Applicants offer the following remarks regarding the rejection as set forth in the Office Action.

The Office Action alleged that "[a]pplicants arguments filed 12/27/2006 have been fully considered but they are not persuasive" (see the Office Action at page 2, line 3). In upholding the rejection, the Office asserted that under 35 U.S.C. §112, first paragraph, it is critical that the "specification discloses a representative number of species to demonstrate that Applicants was in possession of the entire genus at the time the application was filed." The Office stated that "there is no *per se* rule regarding a 'representative number'" and that even though the courts have ruled that under some circumstances a single species is sufficient to describe a broad genus, the determination is "case- and fact-dependent and is related to the predictability of the art." In the case of the instant application, the Office considered the breadth and composition of the genus, the extent to which the distinguishing identifying characteristics of the genus have been disclosed, and the predictability in the art. With respect to the breadth and composition of the genus, the Office alleged that "[t]he claimed genus is exceptionally broad with respect to structure however the genus must also possess as distinguishing functional characteristic, the ability to promote osteoblast proliferation, which narrows the scope of the genus" (see Office Action at page 3).

In response, applicants have removed "analogs" and "fragments of SEQ ID NO:1, 2, or 3" from claims 1, 13, and 25. Applicants have also amended claims 1, 13, and 25 to recite an amino acid sequence that is at least 90% (rather than 60%) identical to SEQ ID NO:1, 2, or 3.

Applicants respectfully submit that the specification discloses distinct preptin peptides and the areas of variability between these peptides (e.g., R_1 to R_9 in formula (I)), as shown at paragraphs [0007] to [0020] of the application. As amended, claims 1, 13, and 25 include peptides that are at least 90% identical to either of these three distinct species of SEQ ID NOs:1-3. Applicants respectfully submit that methods for synthesizing peptides are routine in the art. The specification also discloses the distinguishing functional characteristic of these peptides (i.e., the ability to promote osteoblast proliferation), and an assay for measuring this activity. Thus, applicants respectfully submit that one of skill in the art could, without undue experimentation and inventive skill, easily synthesize any peptide that is at least 90% identical to SEQ ID NO:1, 2, or 3 and assay the activity of the peptide. Applicants respectfully submit, therefore that the specification discloses a representative number of species to demonstrate that applicant was in possession of the entire genus at the time the application was filed, and is adequate to meet the written description requirement for independent claims 1, 13, and 25.

With respect to the extent to which the distinguishing identifying characteristics of the genus have been disclosed, the Office asserted that

> [t]he complete structures of the 512 sequences represented by formula I have been disclosed, including SEQ ID NOs:1, 2, and 3. ... A correlation between the structure of these species and their function has not been presented, rendering it difficult for the skilled artisan to predict in the absence of experimentation, which of the 512 sequences would possess this functional property (see Office Action at page 4)

Applicants have disclosed three different peptides and have provided an alignment of the their sequences to show conserved regions. Given this information, one skilled in the art could easily obtain any species and assay its function using the *in vitro* assay described in the specification. Furthermore, one of skill in the art would reasonably understand that this *in vitro* test could be performed, for example, in a 96-well plate, thus allowing multiple assays to be easily performed simultaneously.

The Office's assertions regarding fragments, preptin analogs, and peptides with at least 60% homology to SEQ ID NOs:1, 2, or 3 are moot in light of the current amendments.

Applicants respectfully submit that, taken together, the embodiments disclosed, the functional limitation, and the assay provided to determine which peptides posses the distinguishing functional characteristic of the genus, are sufficient to fully describe the entire genus.

In addition, the Office asserted that;

> Claims 1, 2, 4, and 6-12 fail to meet the written description requirement for the genus bone condition. The specification defines a bone condition as any disease wherein mediation of osteoblast or osteoclast activity is involved such as osteoporosis, osteopenia and bone defects. Is there any evidence in the prior art for a class of bone diseases that can be treated by targeting this underlying feature? The specification fails to describe the distinguishing characteristics of the entire genus. What patient population should be targeted? What are the symptoms of the diseases and methods of diagnosis? Bone defects in particular is broad and undefined. How is the skilled artisan to recognize which bone defects are related to osteoblast or osteoclast activity and which ones are not? The claims are not supported for the genus bone condition (Office Action at page 7, lines 3 to 12).

Based on the disclosure of the present application, applicants submit that one of skill in the art would clearly recognize that patients suffering from breakdown of bone tissue or bone loss could benefit from treatment with an agent that increases osteoblast activity. The population that should be targeted and the symptoms of the disease and methods of diagnosis would be apparent to one of skill in the art. Nevertheless, to promote clarity and facilitate prosecution applicants have amended claim 1 to read "[a] method for treating a bone condition associated with breakdown of bone tissue or bone loss, ..."

In light of the above remarks and amendments, applicants respectfully submit that the Examiner reconsider and withdraw the rejection under 35 U.S.C. §112, first paragraph.

Enablement

The Office rejected claims 1, 2, 4, 6-14, 16, 18-26, 28, and 30-36 under 35 U.S.C § 112, first paragraph, for allegedly failing to meet the enablement requirement.

The Office Action and the Advisory Action acknowledged that "the specification is enabled for methods comprising the administration of SEQ ID NOs: 1, 2, and 3, fragments comprising residues 17-34 of SEQ ID NOs:1, 2, and 3, but not for the full scope of the claims." As discussed above with respect to written description, the claims, as amended, require peptides comprising an amino acid sequence at least 90% identical to SEQ ID NOs:1, 2, or 3. Thus the claims, as amended, require peptides that fall within the scope of "peptide fragments comprising residues 17-34" of the preptin sequences recited in the specification. Because SEQ ID NOs:1, 2, and 3, have 34 amino acids, the 90% identity limitation in the amended claims permits variability at only two more amino acid positions than a 95% identity limitation. The breadth of the claims is not "excessively broad." It is believed that the amendments overcome this rejection. In addition, applicants offer the following remarks regarding the rejection as set forth in the Office Action.

The Office alleged that "[a]pplicants arguments filed 12/27/2006 regarding the rejection of the claims for failing to comply with the enablement requirement of 35 U.S.C. 112 have been fully considered but they are not persuasive." The Office also discussed the full set of Wands factors, including the state of the prior art and it's predictability or unpredictability, the relative skill of those in the art, the breadth of the claims, the amount of direction or guidance presented and the presence of working examples, and the quantity of experimentation necessary (see Office Action at pages 7 to 13).

With respect to the state of the prior art and its predictability or unpredictability, the Office asserted that "it is unclear how preptin, which acts to stimulate osteoblast proliferation, could treat a disease characterized by overactive osteoblasts" and present Paget's disease as an example of such a disease. The Office also discusses osteoporosis as "a bone conditions that may be treated or prevented by therapies that "act at least in part by preventing osteoblast apoptosis and/or stimulating osteoclast apoptosis." (Jilka et al, Med. Pediatr. Oncol, 2003, 41, 182-5; see also Manolagas, Endocrine Rev., 2000, 21, 115-37)" (see Office Action at pages 8 to 9).

Applicants respectfully submit that as amended, the Office's remarks with respect to Paget's disease are moot, as one of skill in the art would not reasonably understand Paget's disease to be associated with breakdown of bone tissue or bone loss.

As disclosed in the specification, preptin stimulated the proliferation of primary fetal rat osteoblasts and osteoblast-like cell lines, and neonatal calvarial organ culture *in vitro*, and these effects appear to be dependent upon p42/p44 MAP kinase phosphorylation. Preptin is also anti-apoptotic in primary osteoblasts. *In vivo*, preptin stimulates osteoblast proliferation and differentiation, which is shown to significantly increase bone area and mineralizing surface compared to the control in sexually mature male mice (see Table 1 at page 5, and Table 2 at page 6 in the application). Thus, preptin is anabolic to osetoblasts. Preptin does not appear to affect osteoclast development. Applicants respectfully submit, therefore, that one of skill in the art would reasonably understand the therapeutic value of preptin for the treatment of a bone condition associated with breakdown of bone or bone loss.

With respect to the breadth of the claims, the Office discusses the size of the genus prior to the amendments made herein. In light of the amendments, applicants respectfully submit that this rejection is moot.

With respect to the amount of direction or guidance presented and the presence of working examples, the Office alleged that "the specification provides only limited working examples. ... The specification fails to address any address many questions that could guide the skilled artisan" and discusses the structure-function relationship of preptin. In response, applicants respectfully submit that, as amended, the working examples are sufficient to enable the claimed genus.

The Office also asserted that "[t]he specification fails to enable the full scope of the methods ... How is the skilled artisan to recognize conditions that can be treated by the claimed method in the absence of guidance from the specification?" (see Office Action at page 11).

In response, applicants resubmit that one of skill in the art would clearly recognize that patients suffering from breakdown of bone tissue or bone loss could benefit from treatment with an agent that increases osteoblast activity. The Office also asserted that "drugs that inhibit osteoblast apoptosis and promote osteoclast apoptosis are known to treat apoptosis. ... Is it sufficient to target osteoblast apoptosis but not osteoclast apoptosis" (see Office Action at page 11). In response, applicants submit that the Office describes drugs that seem to function solely by modulating osteoblast and osteoclast apoptosis. These examples are not relevant to the instant application, however, as preptin not only inhibits osteoblast apoptosis, but also promotes

osteoblast proliferation and differentiation. Again, the therapeutic potential of preptin, therefore, would be immediately clear to a skilled artisan.

With respect to claims 13, 14, 16, and 18-22, the Office alleged that "[t]he specification presents data suggesting that rat preptin can increase bone area and mineralizing surface of bone. ... Does an increase in bone area or mineralizing surface correlate with an increase in bone density?" (Office Action at page 11, lines 17 to 21).

"Bone area," "mineralizing surface," and "bone density" are art-recognized terms(see Parfitt *et al.*, *Journal of Bone and Mineral Research*, 2: 595-610, 1987; copy attached herewith as Exhibit A). "Bone density," also known as bone mineral density (BMD), refers to a measure of the mass of bone in relation to its volume and/or the volume of calcium and minerals within bone tissue. "Bone area" and "mineralizing surface" are art-recognized bone formation markers. Applicants respectfully submit that increases in bone area and/or mineralizing surface correlate with increasing or maintaining bone density.

With respect to claims 25, 26, 28 and 30-36, the Office asserted that "the specification supports the inhibition of osteoblast apoptosis only" (see Office Action at page 11). In response, applicants have amended independent claim 25 to recite "[a] method for stimulating osteoblast growth or inhibiting osteoblast apoptosis, ..." In view of this amendment, applicants submit that this rejection is moot.

The Office also asserted that "specification provides insufficient guidance on how to select for active preptin peptides and on how to treat all bone conditions, increase bone density and modulate osteoblast apoptosis within the scope of the claims" citing Genetech, 108 F.3d at 1366 (quoting Brenner v. Manson, 383 U.S. 519, 536 (1966) and Rasmusson v. Smithkline Beecham Corp., 75 USPQ2d 1297 (CA FC 2005) (see Office Action at page 12). The Office also alleged that "the skilled artisan would be burdened with undue experimentation in determining if one of the claimed preptin peptides, fragment, analogs or homologs would be effective at treating bone diseases ... The experimentation required represents years of inventive effort and would amount to more of a fishing expedition than routine investigation" (see Office Action at pages 12 to 13).

Applicants submit that these assertions are moot in light of the above amendments and remarks.

In light of the foregoing amendments and remarks, applicants respectfully request reconsideration and withdrawal of the above rejections under 35 U.S.C. §112, first paragraph.

35 U.S.C. § 112, Second Paragraph

The Office rejected claims 2, **24**, and 26 for being allegedly indefinite. However, the Office asserted that "claims 2, **14** and 26 recites the limitation 'the amino acid sequence of preptin' in claims 1,13 and 25, respectively. There is insufficient antecedent basis for this limitation in the claim" (see Office Action at page 13, emphasis added).

Applicants will respond to the rejection with respect to claims 2, 14, and 26. Applicants respectfully submit that, as amended, claims 2, 14, and 26 are cancelled, thus the rejection is moot.

In light of the above amendments and remarks, applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C §112, second paragraph.

CONCLUSION

Allowance of the claims is respectfully requested in view of the above remarks. A Request for Continued Examination, Petition for Extension of Time, and required fees are being filed herewith. Please apply any other charges or credits to deposit account 06-1050, referencing attorney docket no. 11752-010US1.

Respectfully submitted,

Date: December 20, 2007

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Bone Histomorphometry: Standardization of Nomenclature, Symbols, and Units

REPORT OF THE ASBMR HISTOMORPHOMETRY NOMENCLATURE COMMITTEE

A. MICHAEL PARFITT (CHAIRMAN),[1] MARC K. DREZNER,[2] FRANCIS H. GLORIEUX,[3] JOHN A. KANIS,[4] HARTMUT MALLUCHE,[5] PIERRE J. MEUNIER,[6] SUSAN M. OTT,[7] and ROBERT R. RECKER[8]

PRACTITIONERS OF BONE HISTOMORPHOMETRY communicate with each other in a variety of arcane languages, which in general are unintelligible to those outside the field. Many in the bone and mineral scientific community would like to keep abreast of the contributions of histology to their subject, but are dismayed by the semantic barriers they must overcome. The need for standardization has been recognized for many years,[1] during which there has been much talk but no action. To meet the needs of ASBMR members, Dr. B.L. Riggs (President, 1985–1986) asked the senior author to convene a committee of the Society to develop a unified system of terminology, suitable for adoption by the Journal of Bone and Mineral Research as part of its Instructions to Authors. The committee includes members from Europe and Canada as well as the U.S., and represents most existing systems of nomenclature. A circular letter seeking suggestions and information on current usage was sent to several hundred persons, with names drawn from the Society membership roster and lists of attendees at various recent conferences, to which approximately 40 replies were obtained. These confirmed the magnitude of the semantic problem (for some measurements as many as nine different terms were in use) and suggested a range of solutions likely to be generally acceptable.

In formulating the new system, the committee kept in mind certain agreed general principles. First, the primary reason for change was to help other scientists understand bone histomorphometry, not to help bone histomorphometrists understand each other. Second, names should be self-explanatory and descriptive, without implicit assumptions. Third, symbols should consist mainly of abbreviations that included the first letter of each word in the same order as in the name, without subscripts or superscripts. Fourth, each symbol component should have one and only one meaning, and so eliminate ambiguity. Fifth, primary measurements should be clearly distinguished from derived indices. Finally, the chosen system should be sufficiently flexible to apply to all surfaces and all types of bone, and to accommodate any new primary measurement or derived index.

The recommended system shares common elements with, but also differs substantially from, all those in current use, was tested in practice for several months before the final format was chosen, and is as complex and conceptually difficult as the field with which it deals. For those within the field we hope that increased readership of their papers will be adequate compensation for the inconvenience of learning a new system. For those outside the field, mastering the new system will be hard work, but if we are able to secure its acceptance by all journals with an interest in bone and mineral metabolism, the effort will only have to be expended once rather than, as at present, repeated many times. To this end we give the reasons for our decisions in the areas of controversy and, as well as definitions, provide methods for calculation of derived indices and

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their underlying assumptions. For those who wish to use the new system before learning all its details, we provide a summary of its most important components at the end.

PRELIMINARY DEFINITIONS

It is generally agreed that a bone is an individual organ of the skeletal system but the term "bone"† has at least three meanings. The first is mineralized bone matrix excluding osteoid; this usage conforms rigorously to the definition of bone as a hard tissue. Osteoid is bone matrix that will be (but is not yet) mineralized, and is sometimes referred to as pre-bone. The second meaning of "bone," and the one we have adopted, is bone matrix, whether mineralized or not, i.e., including both mineralized bone and osteoid. The third meaning of "bone" is a tissue including bone marrow and other soft tissue, as well as bone as just defined. We refer to the combination of bone and associated soft tissue or marrow as "bone tissue." "Tissue" is defined[2] as "an aggregation of similarly specialized cells united in the performance of a particular function." In this sense bone, bone marrow and the contents of osteonal canals are certainly not the same tissue, but in a more general sense, most textbooks of histology recognize only four fundamental tissues—epithelium, nerve, muscle, and connective tissue,[3] and the last-named includes bone and all its accompanying nonmineralized tissue.

In current clinical and radiologic parlance, "trabecular" and "cortical" refer to contrasting structural types of bone. But "trabecular" does not appear in any standard textbook of anatomy or histology as a name for a type of bone; rather, "spongy" or "cancellous" is used. "Spongiosa" (primary or secondary) is best restricted to the stages of endochondral ossification; "cancellous" is most common in textbooks[3,4] and is the term we have chosen. We retain the noun "trabecula" and its associated adjective "trabecular" to refer to an individual structural element of cancellous bone, in accordance with current practice in histology,[3] pathology,[5] and biomechanics.[6] Etymologically, a trabecula is a beam or rod, and in young persons plates rather than rods are the predominant structural elements, both in the spine[7] and in the ilium,[8] but no convenient alternative is available. An accurate descriptive term for the three-dimensional structure of cancellous bone is "muralium," coined by Elias for the liver;[9] "muralium ossium" is euphonious, but is unsuitable for routine use. The size, shape, and orientation of trabeculae (as just defined) vary considerably between different types of cancellous bone.[8,10]

"Density" is a frequent source of confusion in discussions about bone. We propose that the term should be restricted as far as possible to its primary meaning in physics of mass per unit volume,[11,12] with a subsidiary meaning analogous to population density, which is applied mainly to cells. This precludes the use of "density" in its stereologic sense, as will be discussed later. Corresponding to the definitions given earlier, the volume to which mass is referred can be of mineralized bone, bone, bone tissue (cortical or cancellous), or a whole bone. Mineralized bone density is slightly less than true bone density, which excludes the volume of osteocyte lacunae and canaliculae.[11] This volume is small and generally ignored; lacunar volume can be readily measured,[13] but canalicular volume is inaccessible to light microscopy. Bone density reflects the volumetric proportion of osteoid; bone matrix volume, excluding lacunar and canalicular volume, has been referred to as absolute bone volume.[14] Bone tissue density reflects the volumetric proportion of soft tissue, or porosity. Whole bone density, often referred to as apparent bone density, reflects the volumetric proportions of cortical bone tissue, cancellous bone tissue, and diaphyseal marrow within a bone, whose organ volume is usually measured by Archimedes' principle.[15]

"Osteoblast" is defined differently in the clinical and experimental literature. In young, rapidly growing small animals most bone surfaces are undergoing either resorption or formation and virtually all cells on the surface are either osteoclasts or osteoblasts,[16] but in the adult human, most bone surfaces are quiescent with respect to bone remodeling. We refer to the flat cells that cover quiescent internal (nonperiosteal) bone surfaces as lining cells and restrict the term "osteoblast" to cells that are making bone matrix currently or with only temporary interruption, rather than including all surface cells that are not osteoclasts.[16] Lining cells are of osteoblast lineage and may have osteogenic potential, although this has not been established. The term "osteoclast" is restricted to cells containing lysosomes and acid phosphatase that are resorbing bone; they are usually multinucleated, although some osteoclast profiles may have only one or no nucleus. Criteria for identification of osteoblasts and osteoclasts, whether morphologic or histochemical,[17,18] should always be stated or referenced.

DIMENSIONAL EXTRAPOLATION AND STEREOLOGY

A two-dimensional histologic section displays profiles of three-dimensional structures. Four types of primary measurement can be made on these profiles—area, length (usually of a perimeter or boundary), distance between points or between lines, and number.[19] Some histomorphometrists report all results only in these two-dimensional terms, because the assumptions needed for extrapolation to three dimensions may be difficult to justify and because the diagnostic significance of the measurements or the statistical significance of an experimental result are not affected. For these limited objectives this is a reasonable view, but bone cannot be fully understood unless conceived in three-dimensional terms. In every other branch of science that uses microscopy as an investigative tool, the ultimate goal is to understand three-dimensional reality by the application of stereology, which is the relevant mathematical discipline.[19–21] We believe that this also should be the goal of bone histomorphometry. Accurate three-dimensional data are necessary for proper comparison between species, between bones, and between different types of bone, for input

†We use quotation marks to indicate that only the symbol, not what the symbol refers to, is being considered.

into finite element models of bone strength, for realistic estimation of radiation burdens, and for many aspects of bone physiology, such as the calculation of diffusion distances and the measurement of individual cell work.

But as a practical matter, it is premature to insist on universal adoption of a three-dimensional format. All stereologic theorems require that sampling be random and unbiased, a condition only rarely fulfilled in bone histomorphometry; the closest feasible approach is to rotate the cylindrical bone sample randomly around its longitudinal axis prior to embedding.[19,22] The use of a hemispherical grid[19–21] is a convenient way of ensuring randomness of test line orientation, but cannot compensate for sampling bias introduced at an earlier stage. With the exception of the conversion of area fractions to volume fractions, most stereologic theorems also require that the structure be isotropic, meaning that a perpendicular to any element of surface has an equal likelihood of pointing in any direction in space.[19,23] Although not true for all cancellous bone, in the ilium there is only moderate deviation from isotropy and stereologic theorems may be used with acceptable error.[23,24] But it is more accurate to apply the theory of vertical sections; a cycloid test grid is required, which is incompatible with use of a digitizer,[22,25] but there is no other way of obtaining truly unbiased estimates. Because Haversian canals generally do not deviate from the long axis by more than 10°, stereologic problems in diaphyseal cortical bone are minimal, but investigation of the correct stereologic approach to iliac cortical bone has only just begun.

Accordingly, we recommend that everyone reporting histomorphometric data should select one of two options—either present all results strictly and consistently in two dimensions, using the terms perimeter or boundary (for length), area, and width (for distance), or (as favored by a majority of the committee) present only the corresponding three-dimensional results using the terms surface, volume, and thickness; with the latter option an explanation is needed for each type of measurement of exactly how it was derived from the primary two-dimensional measurement, as described later. A mixture of two- and three-dimensional terms should not be used in the same paper. The only exception is number, the fourth type of primary measurement, for which there is no convenient way of extrapolating to three dimensions without making assumptions concerning the three-dimensional shape of the objects counted.[20,21] Direct enumeration of number in three dimensions is possible if the same object can be identified in serial sections of known thickness and separation,[26] but this method has not yet been applied to bone. Topological properties such as connectivity also cannot be determined from two-dimensional sections.[27]

An important general issue is whether or not to adopt the terminology of the International Society of Stereology, as was suggested at the First International Workshop on Bone Morphometry.[28] Stereologists use the term "density" in a very general sense to identify any measurement referred to some defined containing volume,[20,21] so that fractional volume is "volume density" (V_V) and surface area per unit volume is "surface density" (S_V). Although the unification of scientific terminology is desirable in the long term, the practical disadvantage of using "density" in two different senses appeared to outweigh the theoretical advantage. Furthermore, dislike of stereologic terminology was widespread among the respondents to our questionnaire. Nevertheless, all investigators wishing to remain at the cutting edge of bone histomorphometry will need to be thoroughly familiar with the terminologic conventions of stereology, since many important methodologic papers applicable to bone are now being published in the Journal of Microscopy, which is the official journal of the International Society of Stereology.[25–27]

THE IMPORTANCE OF REFERENTS

Primary two-dimensional measurements of perimeter, area, and number are indices of the amount of tissue examined and can be compared between subjects only when related to a common referent, which will be some clearly defined area or perimeter within the section. Absolute perimeter length and absolute area in two dimensions have no corresponding absolute surface area and absolute volume in three dimensions, but it is convenient to refer to perimeters as surfaces and to areas as volumes if the appropriate referent is clear from the context. Primary two-dimensional measurements of width (and corresponding three-dimensional thicknesses) and mean profile areas of individual structures have meaning in isolation and are the only type that do not require a referent. Different referents serve different purposes and lead to different interpretations, so that use of multiple referents is unavoidable, and it is important to clearly distinguish between them.[29] Commonly used referents include tissue volume (TV), bone volume (BV), bone surface (BS), and osteoid surface (OS) and their corresponding two-dimensional areas or perimeters. With explicit identification of the referent, the use of "relative" as a qualifying term becomes redundant.

The volume of the cylindrical biopsy core is not commonly used as a referent at present, but is needed for comparison with physical methods of measuring bone density,[30] for comparing the absolute amounts of cortical and cancellous bone lost because of aging or disease,[30] for determining the contributions of different types of bone and different surfaces to various histologic indices, such as amount of osteoid and surface extent of osteoblasts,[31] and for examining in detail the relationships between histologic and biochemical indices of whole body bone remodeling.[31] Use of the core volume (CV) as a referent provides the closest approach possible from an iliac biopsy to the *in vivo* level of organization corresponding to bone as an organ. An intact full thickness transiliac biopsy can be regarded as representative of the entire bone,[17,32] since the length of the cylindrical biopsy core perpendicular to the external surface depends mainly on the width of the iliac bone at the site of sampling. With a vertical biopsy through the iliac crest,[4] the proportions of cortical and cancellous tissue in the bone cannot be measured, but with either type of biopsy the results can be weighted by the proportions of cortical and cancellous bone tissue in the entire skeleton.[33] The same principle can be applied to rib biopsies and to long bone cross-sections, by using the whole area enclosed by the periosteum as the referent.

TABLE 1. ABBREVIATIONS AND SYMBOLS OF TERMS USED IN BONE HISTOMORPHOMETRY

A	Apposition(al)	G	Grow(th)(ing)	On	Osteon(al)
Ab	Absolute	H	Haversian	Or	Osteocyt(e)(ic)
Ac	Activation	Hm	Hematopoietic	P	Period
Aj	Adjusted	Hp	Hypertrophic	Pf	Profile
Al	Aluminum	Ht	Height	Pl	Plate
Ar	Area (2D)[a]	Hz	Horizontal	Pm	Perimeter (2D)[a]
a	Activ(e)(ity)	h	Hit	Po	Por(e)(ous)(osity)
B	Bone	I	Interface[c] (3D)[a]	Ps	Periost(eal)(eum)
BMU	Basic Multicellular Unit	Ia	Intra	Pt	Point
Bd	Boundary (2D)[a]	Ic	Intercept	Q	Quiescent
C	Core	Il	Initial	R	Rate
Ca	Canal(icula)(r)	In	Internal	Rd	Radi(al)(us)
Cd	Corrected	Ir	Inter	Rf	Referen(ce)(t)
Ce	Cell	Is	Instantaneous	Rm	Remodeling
Cg	Cartilage	It	Interstitial	Rs	Resorption[d]
Cm	Cement	i	Intersection	Rv	Reversal
Cn	Cancellous	L	Label(led)	S	Surface (3D)[a]
Cp	Cytoplasm(ic)	Lc	Lacuna(r)[f]	Sa	Sample
Ct	Cort(ex)(ical)	Le	Length	Se	Section
Cy	Cycle	Li	Lining	Sg	Sigma
D	Dimension(al)	Lm	Lamella(r)	Sm	Seam
De	Depth	Ln	Line	Sn	Spongiosa
Dg	Degenera(tive)(tion)	Lo	Longitudinal	Sp	Separation
Dm	Diameter	l	lag	St	Structur(e)(al)
Do	Density	M	Mineral(iz)(ing)(ation)	s	Single
Do	Domain	Ma	Marrow	T	Tissue
Dp	Diaphys(is)(eal)	Md	Mineralized	Tb	Trabecula(r)[h]
Dt	Delta	Me	Medullary	Th	Thickness (3D)[a]
d	Double[b]	Ml	Modeling	Tm	Termin(al)(us)
E	Ero(ded)(sion)	Mo	Mononucle(ar)(ated)	Tr	Transitional
Ec	Endocortical	Mp	Metaphys(is)(eal)	Tt	Total
En	Envelope	Mu	Multinucle(ar)(ated)	t	Time
Ep	Epiphys(is)(eal)	Mx	Matrix	U	Unit
Es	Endost(eal)[c](eum)	m	Maturation	V	Volume (3D)[a]
Ex	External	N	Number of profiles or structures	Vd	Void
F	Formation[d]	Nc	Nucle(us)(ar)	Vk	Volkmanns
Fa	Fat(ty)	Nd	Node	Vt	Vertical
Fb	Fibro(sis)(us)	n	Number of sampling units[g]	W	Wall
Fe	Iron	O	Osteoid	Wi	Width (2D)[a]
Fr	Front	Ob	Osteoblast(ic)	Wo	Woven
f	Frequency	Oc	Osteoclast(ic)	Z	Zone

For further definitions and explanations see text.
[a]2D or 3D refers to the format in which data are reported, not the dimensions of an individual quantity.
[b]Also day, but context should eliminate ambiguity.
[c]endocortical + cancellous.
[d]As a process, not as a morphologic feature.
[e]Between osteoid and mineralized bone.
[f]If unqualified, osteocytic, not Howship's.
[g]e.g. subjects, sites, sections, etc.
[h]An individual structure, not a type of tissue.

LEXICON OF BONE HISTOMORPHOMETRY

The recommended individual terms are listed in Table 1 in alphabetical order of their abbreviations or symbols. Several general comments are in order. First, like a dictionary, the lexicon is intended to be consulted rather than memorized. Second, the use of abbreviations is always discretionary, never compulsory. Although designed mainly to save time or space, there is a more subtle reason for abbreviations, as for other symbols. Words frequently carry unwanted implications from their use in other contexts, but confusion is less likely with symbols that can be approached with fewer preconceptions.[1] Nevertheless, our purpose is not to encourage or discourage the use of abbreviations and symbols, but to ensure that the same ones are used by everybody. To this end, we have made the lexicon comprehensive in order to anticipate future needs and forestall the introduction of new abbreviations with different meanings. We have included metals frequently identi-

fied in bone (with their usual elemental abbreviations) and terms commonly used in quantitative microscopy and stereology, as well as terms for all the major structural features of bone and of bones, and for some important concepts of bone physiology. Terms with unfamiliar meanings are explained and defined in relation to their use.

With one exception, the abbreviations and symbols in Table 1 consist of only two letters; "BMU" (Basic Multicellular Unit) has been retained because it is important and widely used and lacks a suitable alternative. The most commonly used descriptive terms are given a single capital letter. Other terms have an additional lowercase letter, chosen in many cases to emphasize the second or later syllable and usually avoiding the second letter of the word abbreviated by the single capital letter. Single lowercase letters are used for terms that are in some sense related to time, for the primary data of classical grid counting (hit and intersection), and for n in its usual statistical sense. When used in combination, double letter abbreviations should be demarcated by a period; in the absence of periods each letter is to be construed as an individual abbreviation. In this way any combination of abbreviations can be unambiguously deciphered without having to determine which terms are included in the lexicon, and even the most abstruse terminology in current use can be translated into the new language with a reduction in mean number of characters per abbreviation or symbol of about 15%.

PROPOSED SYSTEM OF NOMENCLATURE

Bone histomorphometry can be applied to many types of material, but the most common are sections of cylindrical biopsy samples of iliac bone obtained from human subjects, and sections of long bones obtained from experimental animals. For orientation we first present the terminology for describing these sections.

Description of section

"Core" (C) refers to the entire biopsy specimen (Fig. 1). For transiliac biopsies the distance between external (Ex) and internal (In) periosteum is termed "width" (Wi) because it is related to the thickness of the iliac bone at the biopsy site; for vertical biopsies through the iliac crest the term "length" (Le) is more appropriate. Core width is subdivided into cortical (Ct) widths and cancellous (Cn) width; for transiliac biopsies measurements on the two cortices (including their width) are usually pooled, but it is possible to keep track of their identity and examine them separately. The other dimension of the core is referred to as "diameter" (Dm), although only sections through the central axis of the cylinder have the same diameter as the trephine; the more accurate term "chord length" is too cumbersome. If the axis of the transiliac core is oblique to the plane of the ilium, its dimensions are apparently changed (Fig. 2). It is convenient to define core diameter as mean "periosteal length" (external and internal) regardless of obliquity, because true values for cortical and cancellous width corrected for obliquity are then given by the relationships between length and area set out in the legend to Fig. 2.[30,34]

For long bone cross-sections (Fig. 3), bone diameter (B.Dm) is similarly subdivided into two cortical widths and either cancellous diameter (Cn.Dm) for metaphyseal (Mp) cross-sections, or marrow diameter (Ma.Dm) for diaphyseal (Dp) cross-sections. The relationships between these diameters and bone area, cortical area, and cancellous or marrow area depends on the precise geometry of the cross-section. For bio-

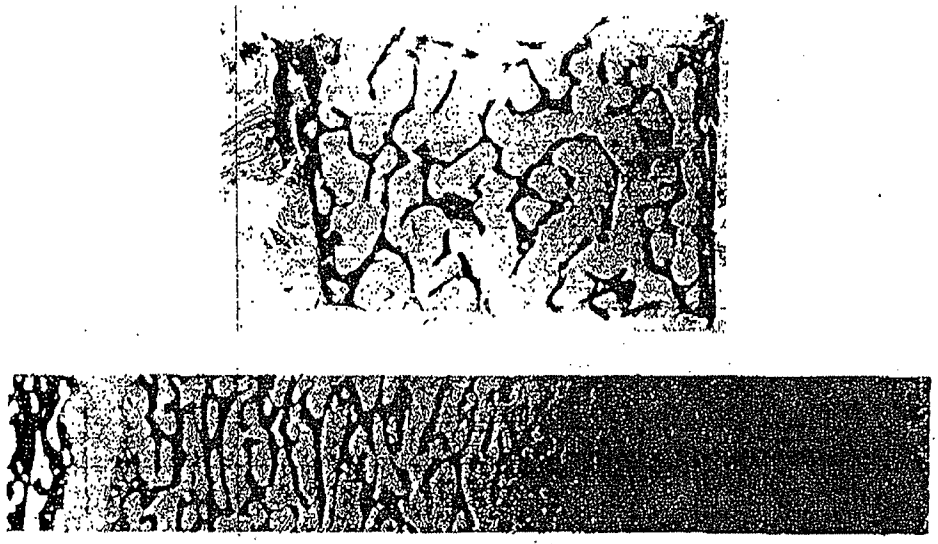

FIG. 1. Sections of representative bone biopsies from different sites. Upper: transiliac (outer cortex on left). Lower: vertical (iliac crest on left). Supplied by H. Malluche; transiliac biopsy reproduced from Ref. 4, with permission.

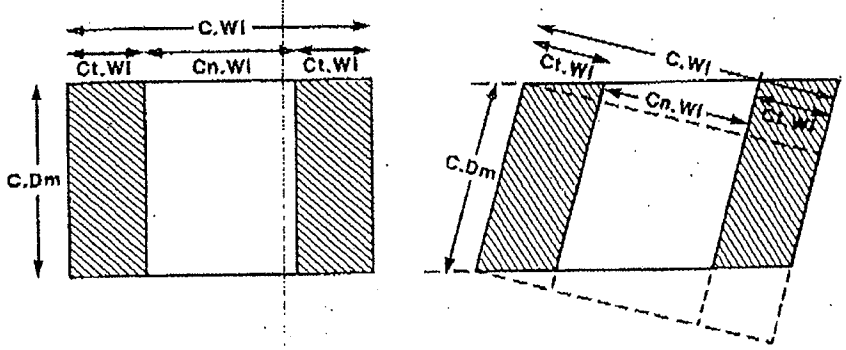

FIG. 2. Diagram of sections through cylindrical biopsy core of ilium. Direction of trephine perpendicular on left, oblique on right. Definitions of abbreviations: C.Wi = core width; C.Dm = Core diameter; Ct.Wi = Cortical width; Cn.Wi = Cancellous width. Relationships to areas: C.Ar = core (or section) area = C.Dm*C.Wi; Ct.Ar = cortical area = C.Dm*Ct.Wi; Cn.Ar = cancellous area = C.Dm*Cn.Wi. Provided the inner and outer periosteum do not depart seriously from parallelism and their mean length is used for C.Dm, these relationships remain true for the oblique section, since the areas enclosed by the interrupted and solid lines are equal.[34] Consequently, the relationships can be used to estimate C.Wi, Ct.Wi, and Cn.Wi without measuring the angle of obliquity.

mechanical purposes such measurements may be needed at multiple locations in relation to the *in vivo* orientation. For both iliac and long bone sections it is necessary for certain purposes to recognize a transitional zone (Tr.Z) lying between cortical and cancellous bone tissue and intermediate in geometrical and topological features.[35] This zone is not indicated in Figs. 2 or 3 because there is not yet a generally accepted method of defining its boundaries. For all bones, all interior surfaces in contact with bone marrow are referred to as endosteal (Es) and are subdivided into cancellous bone surface and endocortical (Ec) surface; the latter is the inner boundary of the cortex. Demarcation between these components is subject to large observer error[36] unless made in accordance with some well-defined rule[37] and will also depend on whether the transitional zone is measured separately. Interior surfaces not in contact with bone marrow are generally referred to as cortical (Ct), with optional qualification as "intra" (In); the cortical surface can also be referred to as the haversian canal (H.Ca) or osteonal canal (On.Ca) surface.

Standard format

We propose a standard and universally applicable method for reporting all data:

Source — Measurement/Referent

Note that the complete elimination of ambiguity applies to punctuation as well as to terminology; the dash (—) and slash (/) are used only as illustrated and periods are used only as described earlier. "Source" refers to the structure on which

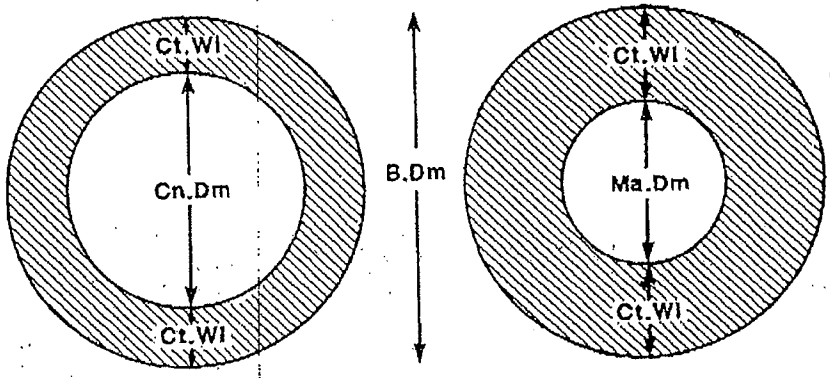

FIG. 3. Diagram of cross-sections through the shaft of a long bone; metaphseal region on the left, diaphyseal region on the right. For clarity, the cancellous bone of the metaphysis is not shown. Definitions of abbreviations: B.Dm = bone diameter; Ct.Wi = cortical width; Cn.Dm = cancellous diameter; Ma.Dm = marrow diameter.

TABLE 2. SOURCES AND REFERENTS IN BONE HISTOMORPHOMETRY

Sources		*Referents*	
Name	*Abb.*	*Name*	*Abb.*
Total core	Tt	Bone surface	BS
Cortical bone tissue	Ct	Bone volume	BV
Cancellous bone tissue	Cn	Tissue volume	TV
Endocortical surface	Ec	Core volume	CV
Periosteal surface	Ps	Osteoid surface	OS
Transitional zone	Tr.Z	Bone interface	BI
Diaphyseal bone	Dp	Eroded surface	ES
Metaphyseal bone	Mp	Mineralized surface	Md.S
Epiphyseal bone	Ep	Osteoblast surface	Ob.S
Medullary bone	Me	Osteoclast surface	Oc.S

Abb. = abbreviation. Those listed will cover most situations in both human and nonhuman studies, but neither list is exhaustive. Combinations of source terms may be needed, such as Dp.Ec for diaphyseal bone, endocortical surface.

the measurement was made, whether this was a particular surface or a particular type of tissue. Most of the commonly used sources have already been defined (Table 2); many others are definable by using the lexicon (Table 1). If measurements are restricted to some subdivision of a source, such as the outer portion of a cortex[31] or the central zone of cancellous tissue,[32] the same symbol can be used, but the appropriate qualification should be made in the description of methods. For measurements made on the entire section, the source is identified as "total" (Tt). Usually it will not be necessary to specify the source each time a particular quantity is referred to—if only one source is used in a paper, it need only be mentioned once. If several sources are included, their names can be used as subheadings for presentation of results in tables or text, and in most cases will need to be repeated only if measurements from several sources are discussed together, such that confusion between them is possible. For some measurements, such as trabecular thickness, only one source is possible and its specification is redundant.

The need for referents was described earlier. The most commonly used referents have already been defined and are listed in Table 2, but the relationships between them need further explanation, as follows:†

$$/BS*BS/BV = /BV$$
$$/BS*BS/TV = /TV = /BV*BV/TV$$
$$/BS*BS/CV = /CV = /BV*BV/CV$$

The three surface/volume ratios and the two volume/volume ratios are the key quantities needed to convert from one referent to another.[29] BS/BV is equivalent to S/V in stereologic terminology, and BS/TV and BS/CV are equivalent to S_V (surface density) in stereologic terminology. These ratios are derived from the corresponding two-dimensional perimeter/area ratios—B.Pm/B.Ar, B.Pm/T.Ar and B.Pm/C.Ar—by multiplying either by $4/\pi$ (1.273), which is correct for isotropic structures,[19-21] or by 1.2, which has been experimentally determined for human iliac cancellous bone.[24] The ratios increase with microscopic resolution, so that the magnification must always be stated and preferably standardized.[39] BV/TV and BV/CV correspond to V_V (volume density) in stereologic terminology and are numerically identical with the corresponding area/area ratios B.Ar/T.Ar and B.Ar/C.Ar.[19-21]

For some purposes a subdivision of the bone surface is needed as a referent (Table 2). Osteoblast surface (Ob.S) and mineralizing surface (MS) are often related to osteoid surface (/OS). Osteoclasts usually avoid osteoid and it can be useful to relate osteoclasts to the mineralized surface (/Md.S), previously called nonosteoid surface,[40] as an alternative to the more usual referents bone surface and eroded surface (/ES). Various kinetic indices of bone formation can be related to the osteoblast surface (/Ob.S) or to the number of osteoblast profiles (/N.Ob), as well as to osteoid surface or bone surface.[29] Finally, it may be apropriate to use the interface between mineralized bone and osteoid, or bone interface, as a referent (/BI) for the length of tetracycline label or of positive aluminum staining, since the interface is where these features are located. In many cases, as when only one referent is used for each measurement, the referent need only be specified once and not repeated each time the measurement is mentioned. If more than one referent is used, measurements with the same referent can be grouped together to avoid repetition.

Primary measurements

These are listed together with abbreviations in both 3D and 2D form in Table 3. Many have already been defined but some need additional explanation.

Area measurements: "Mineralized volume" is used for simplicity instead of mineralized bone volume, and is given by (bone volume − osteoid volume). Osteoid may need to be qualified as lamellar, OV(Lm), or as woven, OV(Wo). Note the distinction in the lexicon between M, which refers to a process, and Md, which refers to a state; for convenience all

†The asterisk (*) is the most typographically convenient symbol for multiplication.

TABLE 3. PRIMARY MEASUREMENTS IN BONE HISTOMORPHOMETRY

Type of measurement	Name of measurement	Abbreviations 3D	Abbreviations 2D
I. *Area*	Bone volume[a]	BV	B.Ar
	Osteoid volume	OV	O.Ar
	Mineralized volume	Md.V	Md.Ar
	Vbid volume	Vd.V	Vd.Ar
	Marrow volume	Ma.V	Ma.Ar
	Fibrosis volume	Fb.V	Fb.Ar
	Canal volume[b]	Ca.V	Ca.Ar
	Cell volume[b,c]	Ce.V	Ce.Ar
	Cytoplasmic volume[b,d]	Cy.V	Cy.Ar
	Nuclear volume[b,d]	Nc.V	Nc.Ar
II. *Length*	Bone interface[e]	BI	B.Bd
	Bone surface[f]	BS	B.Pm
	Osteoid surface	OS	O.Pm
	Eroded surface	ES	E.Pm
	Quiescent surface[g]	QS	Q.Pm
	Mineralized surface[h]	Md.S	Md.Pm
	Osteoblast surface	Ob.S	Ob.Pm
	Single-labeled surface[i]	sLS	sL.Pm
	Double labeled surface[i]	dLS	dL.Pm
	Osteoclast surface	Oc.S	Oc.Pm
	Reversal surface[j]	Rv.S	Rv.Pm
III. *Distance*[k]	Cortical thickness[l]	Ct.Th	Ct.Wi
	Wall thickness	W.Th	W.Wi
	Mineralized thickness	Md.Th	Md.Wi
	Osteoid thickness	O.Th	O.Wi
	Label thickness	L.Th	L.Wi
	Trabecular thickness[m]	Tb.Th	Tb.Wi
	Interstitial thickness	It.Th	It.Wi
	Trabecular diameter[n]	Tb.Dm	—[o]
	Canal radius	Ca.Rd	—[o]
	Cell height[c]	Ce.Ht	—[o]
	Nuclear height[d]	Nc.Ht	—[o]
IV. *Number*[p]	Osteoblast number	—	N.Ob
	Osteoclast number	—	N.Oc
	Osteocyte number	—	N.Ot
	Nuclear number[d]	—	N.Nc
	Canal number	—	N.Ca
	Seam number	—	N.Sm
	Erosion number	—	NE
	Profile number	—	N.Pf
	Node number	—	N.Nd
	Terminus number	—	N.Tm

[a]Area in 2D.
[b]Potential confusion between tissue aggregates and individual structures; see text.
[c]Specify cell type if needed, e.g., Oc.V or Oc.Ar.
[d]Qualify by cell type if needed, e.g., Oc.Nc.V.
[e]Boundary in 2D.
[f]Perimeter in 2D.
[g]BS − (OS + ES).
[h]ES + QS.
[i]Alternative terms are single (or double) labeled interface (sLI, dLI).
[j]ES − Oc.S.
[k]Between points or lines.
[l]Width in 2D; for the cortex, width and thickness are numerically equal, but for other measurements, thickness = width divided by $4/\pi$ or by 1.2.
[m]Assumes that trabeculae are thin plates; $^{(51)} = 2/(BS/BV)$.
[n]Assumes that trabeculae are cylindrical rods; $^{(52)} = 4/(BS/BV)$.
[o]No unique corresponding term in 2D.
[p]No 3D equivalent by standard methods; with appropriate referent could be referred to as density. For further details see text.

tetracycline-based measurements are considered with the kinetic indices discussed earlier. "Void"† is a general term applicable to all tissue that is not bone[41] and includes marrow in cancellous bone and Haversian and Volkmann canals in cortical bone. For both types of tissue: porosity (Po) = void volume/tissue volume.

Problems can arise with area measurements on individual profiles, such as cells or cortical canals. The profiles can be treated as an aggregate of tissue, indicated by use of the appropriate referent. For example, Cc.V/TV is the total area of all cell profiles referred to the total area of tissue and expressed in 3D terms. The profiles can also be treated as individual structures, indicated by absence of a referent; e.g., Ca.Ar is the mean area of individual canal profiles. If confusion is still possible, the term could be qualified as total (Tt) or mean ($\bar{x}$). Mean areas in 2D cannot be extrapolated to mean volumes in 3D unless the structures are counted in 3D.[26] Assuming cylindrical geometry, mean canal area can be used to estimate canal radius (Ca.Rd), but it is preferable to measure this directly, as described later.

Perimeter measurements: Osteoid seams do not end abruptly so that some minimum width should be specified for measurement of osteoid surface (OS). We avoid the terms formation (or forming) surface and resorption (or resorbing) surface because the implications of current activity may be erroneous, and for the same reason we avoid the qualification "active." Eroded surface (ES) is synonymous with crenated or lacunar surface and comprises the osteoclast surface (Oc.S) and the reversal surface (Rv.S); individual erosions can also be classified as osteoclast positive, ES(Oc+), or osteoclast negative, ES(Oc−). Some mononuclear cells probably resorb bone[42] and better methods are needed for identifying and classifying the nonosteoclast cells on the eroded surface, or reversal cells. Quiescent surface (QS) is synonymous with resting or inactive surface; the term implies that remodeling activity will return at some future time. The thin layer of unmineralized connective tissue lying beneath the flat lining cells on quiescent surfaces should not be referred to as osteoid.[43] It is possible that some eroded surface covered by flat lining cells should be counted as quiescent surface rather than as reversal surface.

Distance measurements: In principle, all distance measurements can be obtained in two ways—either by direct measurement at multiple locations or by indirect calculation from measurements of area and perimeter. The direct method is more precise and can provide a frequency distribution and a standard deviation as well as a mean value but requires that measurement sites be randomly selected.[44] The indirect method is less laborious and less subject to sampling bias. The direct method is usually used for wall thickness, distance between labels, and cell and nuclear dimensions; and the indirect method is usually used for trabecular thickness (plate model), diameter (rod model), and separation. Both methods are widely used for osteoid thickness and cortical thickness. The direct method is essential for reconstructing the remodeling sequence from the relationships between individual measurement values at particular locations and instantaneous values at particular times during the remodeling cycle.[42,45] The mean value determined by either method in an individual must be distinguished from the mean value in a group of subjects.

Mineralized thickness is the distance from the cement line to the interface between bone and osteoid.[45] It is used in remodeling sequence reconstruction[42] and in characterizing different types of abnormal osteoid seam, and defining different stages of severity in osteomalacia;[46] the mean value should be close to the difference between wall thickness and osteoid thickness. Label thickness is measured on an individual label; it has been used in the rat for calculation of the rate of initial mineral accumulation[47] and in human subjects as an index of treatment response in renal osteodystrophy.[48] Interstitial thickness (It.Th) is the mean distance between cement lines on opposite sides of a trabecula, usually calculated as Tb.Th-2*W.Th for the plate model.[49] Canal radius is an index of bone loss from the cortical surface, but too little is known of the internal geometry of iliac cortical bone to decide the most stereologically correct method of measurement. On the reasonable but unproven assumption that elliptical profiles are the result of oblique sections through cylindrical canals, direct measurements can be restricted to the short axes of the ellipses.[50]

Number measurements: Most of these are self-explanatory, but restriction to 2D and invariable need for a referent must be re-emphasized. In most cases the referent will be an area or perimeter, but number of nuclei can also be expressed per cell; e.g., N.Nc/Oc is the mean number of nuclear profiles per osteoclast profile. Profile number without qualification refers to isolated bone profiles in cancellous bone tissue, a quantity that increases with age as connectivity declines, and then decreases as some remaining structures are completely removed. Nodes are branch points and termini are end points in a trabecular network that has been skeletonized to facilitate examination of its topological properties.[53] Termini are usually referred to as free ends, but this term is less convenient to abbreviate in the lexicon. The ratio of nodes to termini (Nd/Tm) in a section is an index of spatial connectivity.[54]

Derived indices

These can be either structural or kinetic (Table 4). Many of the calculations are based on assumptions that are reasonable but not rigorously established, and individual investigators may decide to use all, some, or none of the indices that we have selected.

Structural indices: Trabecular number (or density) is usually calculated with dimensions Length^{-1}† according to the parallel plate model as (BV/TV)/Tb.Th, which is numerically equal to one-half of BS/TV for cancellous bone.[55] With the alternative cylindrical rod model[52] Tb.N is given with dimensions Length^{-1} by $(4/\pi * BV/TV)^{0.5}$/Tb.Dm. To maintain consistency between the alternative models, this is preferred to the corresponding squared value with dimensions Length^{-2}. Trabecular separation, defined as the distance between edges rather than between mid points, is calculated according to the

†In specifying dimensions, length and time are usually abbreviated L and T, but these have other meanings in the lexicon.

TABLE 4. DERIVED INDICES IN BONE HISTOMORPHOMETRY

Type of index	*Name of index*[a]	*Abbreviation*[a]	*Formula*[b]
I. *Structural*	Trabecular number	Tb.N	(BV/TV)/Tb.Th[c]
	Trabecular separation	Tb.Sp	(1/Tb.N) − Tb.Th[c]
II. *Kinetic*	Mineralizing surface[d]	MS	(dLS + sLS/2)/BS[c]
	Mineral apposition rate	MAR	Ir.L.Th/Ir.L.t
	Adjusted apposition rate[e]	Aj.AR	MAR*(MS/OS)
	Osteoid apposition rate	OAR	same[g]
	Mineral formation rate[d]	MFR	MAR*(MS/BS)
	Bone formation rate[d]	BFR	same[g]
	Bone resorption rate[d]	BRs.R	see text
	Mineralization lag time	Mlt	O.Th/Aj. AR
	Osteoid maturation time	Omt	O.Th/MAR[h]
	Formation period	FP	W.Th/Aj.AR
	Resorption period	Rs.P	FP*(Oc.S/OS)[h]
	Reversal period	Rv.P	FP*(ES − Oc.S)/OS
	Remodeling period[i]	Rm.P	FP*(ES + OS)/OS
	BMU lifespan (sigma)	Sg (or σ)	see text
	Quiescent period	QP	FP*(QS/OS)
	Total period[j]	Tt.P	FP*(BS/OS)
	Activation frequency[k]	Ac.f[l]	(1/Tt.P)

[a]Name and abbreviation are the same whether 2D or 3D expression used, except for mineralizing surface.
[b]For 3D expression; in applying these formulae it is essential to keep track of units throughout the calculations.
[c]For parallel plate model; see text for rod model.
[d]Referent must be specified; /BS is used in formula.
[e]Other methods of measurement and calculation can be used (see text).
[f]Time averaged over osteoid seam life span.
[g]Mean value given by preceding quantity in steady state and in absence of osteomalacia.
[h]For more accurate method see Ref. 42.
[i]Rs.P + Rv.P + FP.
[j]Rm.P + QP.
[k]1/Tt.P.
[l]An alternative to μ.

parallel plate model as Tb.Th*(TV/BV-1), or as (1/Tb.N)-Tb.Th. This quantity when multiplied by $\pi/2$ is an estimate of the mean distance across marrow cavities.[19,23] According to the cylindrical rod model, and assuming a parallel rectangular lattice, trabecular separation is given by Tb.Dm*((π/4*TV/BV)$^{0.5}$-1) but cannot be related in any simple way to the size of the marrow cavities. Trabecular spacing, defined as the distance between midpoints, is given by 1/Tb.N, and can also be measured directly.[56]

Mineralizing surface: The extent of surface active in mineralization at a particular time is given by the total extent of the labeled surface resulting from label administration at that time. The total extent of double label plus half the extent of single label is equivalent to the mean of the separately measured first label length (L1) and second label length (L2), thus following the normal scientific procedure of taking the mean of two separate observations when they are available. Use of the neutral term mineralizing surface (MS) or mineralizing interface (MI) allows a choice between the mean of the two labels, the second label alone (because it is closer in time to the biopsy), the total label (if only one label was given), *in vitro* tetracycline staining,[57] histochemical identification of the mineralization front,[40] or autoradiography after radiocalcium administration. Whatever the choice, the specification and validation of the method and of the exact conditions of measurement are the responsibility of the investigator. MS can be expressed in relation to a variety of referents (Table 2); MS/OS is equivalent to the fraction of osteoid seam life span during which mineralization occurs.

Apposition rates: Mineral apposition rate (MAR) is the distance between the midpoints[29] or between the corresponding edges[58] of two consecutive labels, divided by the time between the midpoints of the labeling periods. Both the number of sites available for measurement and the mean value of the measurement may vary with the length of the labeling interval,[29,58] which must always be stated and preferably standardized. We avoid the terms calcification rate and mineralization rate, since they may lead to confusion between mineral apposition and mineral accumulation[59] and are often used in radiocalcium kinetics to refer to the whole body bone formation rate. There is no convenient way of distinguishing between the two-dimensional and three-dimensional quantities by

different names, so that if the latter is chosen, it is important that the dimensional extrapolation factor be used consistently. Adjusted apposition rate (Aj.AR) is calculated as MAR*MS/OS, and represents either the mineral apposition rate or the bone formation rate averaged over the entire osteoid surface.[59,60] It is analogous to the osteon radial closure rate[61] and is synonymous with effective apposition rate,[62] corrected apposition rate,[63] formation velocity,[64] and "bone formation rate—BMU level—surface referent,"[60] but none of these alternative names is satisfactory.

The concept is important because in a steady state and in the absence of osteomalacia the adjusted apposition rate is the best estimate available from a biopsy of the mean rate of osteoid (or matrix) apposition. Under these conditions the rates of formation of mineralized bone and of bone matrix, time-averaged over the osteoid seam life span, including periods of activity and inactivity, are identical even though their instantaneous values are systematically out of step,[59] and the term osteoid apposition rate (OAR) may be used. We refer to these quantities (Aj.Ar and OAR) as apposition rates rather than as formation rates in order to maintain the distinction that an apposition rate has meaning at a point on the surface, whereas a formation rate has meaning only in relation to some aggregate of tissue, either surface or volume. An apposition rate represents in some sense the activity of a team of osteoblasts, but a formation rate is influenced by the rate of remodeling activation and so depends on the number of osteoblast teams as well as on their activity. The team rather than the single cell is emphasized as the conceptual unit, since the activity of the team depends on the number of its members as well as on their individual productivity.

Formation and resorption rates: Mineral formation rate (MFR) is the volume of mineralized bone formed per unit time, calculated as the product of mineral apposition rate and mineralizing surface as defined earlier. If this term could be misinterpreted as relating to the physical chemistry of mineralization, the more precise term mineralized bone formation rate (Md.BFR) can be used. In a steady state and in the absence of osteomalacia the mineral formation rate is identical with the bone formation rate (BFR), and except when the distinction is important, the latter and more familiar term should be used. There is a bone formation rate corresponding to each possible referent for mineralizing surface: /OS, /BS, /BV, /TV, and /CV. Bone formation rate calculated using the osteoid surface referent is numerically identical to the adjusted apposition rate, as explained earlier. Expressing bone formation rate per unit of bone surface (BFR/BS) seems most logical when considering hormonal effects on bone remodeling.[31] Bone formation rate per unit of bone volume (BFR/BV) is equivalent to the bone turnover rate, which determines bone age and various age-dependent properties of bone.[59] Bone formation rate per unit of tissue volume (BFR/TV) seems most logical when considering biochemical markers of bone remodeling, since the entire tissue is perfused and contributes its products to the circulation.[31] The significance of the core volume referent was discussed earlier.

Bone resorption rate (BRs.R) cannot be measured directly by histomorphometry but can be estimated indirectly as the bone formation rate increased or decreased by an assumed or measured rate of change of bone volume, provided that all terms are expressed in relation to the same referent.[29,65,66] Previous gains or losses of bone from a surface can be estimated by comparing trabecular thickness and number, cortical thickness and osteonal canal radius with mean values in age-matched control subjects, but it cannot be assumed that bone formation persisted at the current rate throughout the time over which these changes occurred. Since the rate of bone loss rarely exceeds 10% of the rate of bone turnover, under most circumstances the error from assuming that resorption and formation rates are equal is less than the error of measurement, but it is more accurate to assume that mineralized volume changes in proportion to some local or whole body measurement of bone mineral.[66] An alternative is to use sequential biopsies to estimate the change in bone volume,[29] which is satisfactory for a group of adequate size, but subject to substantial error from sampling variation in a single subject. However it is estimated, BRs.R can be expressed in relation to a variety of different referents, including osteoclast number.[66]

Timing of mineralization: Mineralization lag time (Mlt) is the mean time interval between deposition and mineralization of any infinitesimal volume of matrix, averaged over the entire life span of the osteoid seam, and is given by O.Th/Aj.AR. The concept is important in the understanding of osteomalacia and the control of osteoid volume, since it can be demonstrated that OV/BV = BFR/BV*Mlt,[46] corresponding respectively to the birth rate and life span of individual moieties of osteoid.[59] Mlt must be distinguished from osteoid maturation time (Omt), which is the mean time interval between the onset of matrix deposition and the onset of mineralization at each bone forming site. The name implies that the delay results from extracellular modification of the matrix, such as collagen cross-linking.[59] In the growing rat Mlt and Omt are identical, but in human subjects Omt is usually shorter and never longer than Mlt. Omt can be estimated as O.Th/MAR, and has also been referred to as direct rather than indirect Mlt,[67] but it is more accurate to measure Omt by remodeling sequence reconstruction.[42] Omt provides less insight into the mechanisms of osteoid accumulation than Mlt, but it may be more convenient for diagnostic use since, unlike Mlt, it is always normal in osteoporosis.[46]

Remodeling cycle duration and its subdivisions: Formation period (FP) is the mean time required to rebuild a new bone structural unit (B.St.U) or osteon from the cement line back to the bone surface at a single location, and is given by W.Th/Aj.AR. It includes so-called downtime or offtime[61] or whatever other mechanism contributes to the difference between osteoid surface and mineralizing surface that cannot be attributed to label escape,[60] and so can be qualified as active, FP(a+), given by W.Th/MAR, or inactive, FP(a−), given by W.Th/Aj.AR*(OS/MS-1), or FP-FP(a+). FP(a+) has also been referred to as osteoblast life span.[69] FP is the key quantity needed for calculation of all other temporal subdivisions of the remodeling sequence. In a steady state, fractions of space are equivalent to fractions of time,[59] so that xP = xS/OS*FP, where x is any remodeling state other than formation, in-

cluding osteoclastic resorption, reversal, and quiescence (Table 4), but these calculations will reflect the uncertainty in classifying reversal cells.[42,58] Osteoclasts are motile and their area of activity probably extends beyond their current contact area[59] and in principle the osteoclast domain (Oc.Dm) determined by scanning electron microscopy[70] could be used to calculate RP.

The sum of the resorption, reversal, and formation periods is the remodeling period (Rm.P), which is the average total duration of a single cycle of bone remodeling at any point on a bone surface. Rm.P is substantially shorter (by a factor of 2 or 3) than the total duration of bone remodeling activity that follows a single event of activation, because once initiated, the remodeling process moves for a variable distance across the bone surface or through the bone.[59] For example, many cortical osteons are much longer than a single cortical BMU, including both cutting and closing cones,[59] and the three-dimensional extent of many trabecular osteons is much larger than the extent of a single erosion or a single osteoid seam.[71] Although not commonly recognized, it is this extended period[59] which is the true BMU life span (or sigma) needed for attainment of a new steady state after any pathogenetic or therapeutic intervention.[61] Although σ remains an acceptable symbol for this crucially important concept, Sg is an alternative that avoids the inconvenience of Greek letters that is experienced with most typewriters and printers.

Activation interval and frequency: The sum of the remodeling period and the quiescent period (QP) is the total period (Tt.P), which is the average time interval between the initiation of two successive remodeling cycles at the same point on the surface.[42,59] The reciprocal of Tt.P is the activation frequency (Ac.f), which is the probability that a new cycle of remodeling will be initiated at any point on the surface by the event of activation.[42,59] Ac.f can also be calculated in the more traditional manner as the birth rate of remodeling sites of assumed or measured mean area,[59] and expressed in relation to the various volume referents in Table 2. We do not use the traditional symbol μ for this quantity, because in addition to the inconvenience of a Greek letter, there is possible confusion with μm as a unit of length, and the anglicized version mu can be confused with Mu as an abbreviation for multinucleated (Table 1) and with MU as an abbreviation for mechanical usage.[72] It can be shown that Ac.f*W.Th = BFR/BS, which is reasonable, since W.Th can be regarded as the average amount of bone formed per activation event.

Units and dimensions

We propose the use of two primary units of length, micrometer (mcm) and millimeter (mm), and two primary units of time, day (d) and year (y), with the choice depending on convenience, consistency, and the principle of providing the most important information in front of rather than after the decimal point. Dimensions are useful for checking equations and derivations[73] and for indicating the similarities between some quantities expressed in different units. For surface/surface and volume/volume ratios, we prefer percentages rather than decimal fractions, as did most of the respondents to our questionnaire; in this case the percent sign can be used to combine the referent and unit (e.g., OS% BS instead of OS/BS(%)). If abbreviations are not used for these ratios, the names can be simplified by writing the type of measurement only once (e.g., osteoid/bone (surface)). We avoid units such as mm^2/cm^2, since their magnitude changes with transition from two to three dimensions (e.g., 1 mm^2/cm^2 = 10 mm^3/cm^3). Such units also do not conform to the SI[74] and make it more difficult to perceive that the quantity is dimensionless. All section dimensions should be expressed in mm, all primary perimeter and area measurements in mm or mm^2, and all surface/volume ratios in mm^2/mm^3 ($Length^{-1}$). Thickness measurements should be expressed in mcm, with mm as an alternative for cortical thickness. Apposition rates should be expressed as mcm/d ($Length*Time^{-1}$) and formation rates with volume referent as %/y ($Time^{-1}$). Times and periods should be expressed in d or y as most appropriate and activation frequency in /y ($Time^{-1}$).

SUMMARY OF NEW SYSTEM OF NOMENCLATURE

We recognize that many persons who perform bone histomorphometry or interpret its results will on most occasions need to use only a small proportion of the foregoing material. To facilitate access to the new system we provide a summary of its most important features, but this is not intended to stand on its own without reference to the main body of the paper.

Definitions

All acceptable terms are listed in Table 1; only the most basic are discussed here. The term "bone" refers to bone matrix whether mineralized or not and "bone tissue" refers to bone as defined with its associated marrow or other soft tissue. Bone tissue is usually either cortical or cancellous; the junction between them, which is the inner border of the cortex, was previously referred to as "inner cortical" or "cortical-endosteal" surface, but is now referred to as "endocortical surface." A trabecula is an individual structural element of cancellous bone tissue, whether platelike or rodlike in form. The term "osteoid" refers to unmineralized bone matrix that in the normal course of events will become fully mineralized, and does not include the thin layer of permanently unmineralized collagen-containing connective tissue that lies beneath bone lining cells on all quiescent surfaces. The junction between osteoid and mineralized bone is referred to as the "bone interface" or, more precisely, "osteoid-bone interface."

The term "osteoblast" is restricted to cells that are currently making bone and does not refer to all cells with osteogenic potential. The qualifications "active" and "inactive" are not used; "inactive osteoblasts" are called "lining cells." Terms that embody assumptions, such as "formation (or forming) surface" and "resorption (or resorbing) surface," are avoided. Instead, the purely descriptive terms "osteoid surface" and "eroded surface" are used. The extent of currently active mineralization is referred to as "mineralizing surface" (or interface) regardless of how it is estimated, a term that replaces "labeled surface" or "calcification front." The method used

for its determination must be specified and justified. A cylindrical biopsy specimen from the ilium, whether transverse or vertical, is referred to as a "core" and the term "total" is generally used only when measurements are made on the entire core.

General principles

Dimensional expression: There must be consistent use of only two-dimensional or only three-dimensional terminology and units throughout the same paper or the same report. Primary measurements are referred to as area, perimeter (or boundary), and width if expressed in two dimensions and as volume, surface (or interface), and thickness if expressed in three dimensions (Table 1). Number, the fourth type of primary measurement, can be expressed three-dimensionally only if serial sections are examined. If three-dimensional expression is used, the method of calculation should be exactly specified and its underlying assumptions carefully considered.

Stereology: The terminology and symbols of the International Society of Stereology will not be used. Consequently, the term "density" retains its primary meaning in physics of mass per unit volume. However, this in no way diminishes the

TABLE 5. COMPARISON OF NEW WITH OLD TERMINOLOGY FOR SELECTED PRIMARY MEASUREMENTS (UPPER LIST) AND DERIVED INDICES (LOWER LIST) ON CANCELLOUS BONE TISSUE

Present terminology[a]	*Proposed terminology*[b,c]	*Abbreviation*	*Units*
Trabecular[d] bone volume (TBV)	Bone volume[e]	BV/TV[e]	%
(Relative) osteoid volume (ROV)	Osteoid volume	OV/BV	%
(Absolute) osteoid volume[f] (AOV)	Osteoid volume	OV/TV	%
(Relative) osteoid surface (ROS)	Osteoid surface	OS/BS	%
(Active[g]) osteoblast surface (AOS)	Osteoblast surface	Ob.S/BS[h]	%
(Mean[i]) osteoid seam width (MOSW)	Osteoid thickness	O.Th	mcm
(Total) resorption surface[j] (TRS)	Eroded surface	ES/BS	%
(Active[k]) resorption surface (ARS)	Osteoclast surface	Oc.S/BS[l]	%
Osteoclast index (OI)	Osteoclast number	N.Oc/T.A[m]	/mm^2
(Trabecular) Specific surface[n] (tS_{sp})	Bone surface	BS/TV	mm^2/mm^3
Active[o] forming surface[p] (AFS)	Mineralizing surface	MS/BS	%
Mineralization[q] front (MF)	Mineralizing surface	MS/OS	%
Calcification[r] rate (CR)	Mineral apposition rate	MAR	mcm/d
Mean[i] trabecular[s] plate thickness (MTPT)	Trabecular thickness	Tb.Th	mcm
Mean[i] trabecular[s] plate density (MTPD)	Trabecular number[t]	Tb.N	/mm
Mean[i] trabecular[s] plate separation (MTPS)	Trabecular separation[t]	Tb.Sp	mcm
Bone formation rate, BMU level[u] ($sV_{(BMU)}$)	Adjusted apposition rate	Aj.AR	mcm/d
Bone formation rate, tissue level (^tV_f)	Bone formation rate	BFR/BS	mcm^3/mcm^2/d
Bone formation rate, volume referent[v] (^vV_f)	Bone formation rate	BFR/BV	%/y

[a]These are representative of current practice in different laboratories; it is not implied that all are used by any laboratory or that any are used by most laboratories. Qualifying terms are in parentheses if their use is inconsistent between laboratories.
[b]Measurement name only; need for inclusion of source and/or referent in name varies with context, as discussed in text.
[c]Three-dimensional expression except where otherwise stated.
[d]Source almost always included in name for this quantity, often omitted for others.
[e]The full name and abbreviation would be cancellous bone volume/tissue volume (Cn-BV/TV); see notes b and d.
[f]Also called osteoid volume density.
[g]Designation usually based on morphology.
[h]OS is another frequently used referent.
[i]Including "mean" as part of the name should imply direct rather than indirect measurement and may lead to confusion with the mean value in a group of subjects.
[j]Also termed crenated or Howship's lacunar surface.
[k]Designation usually based on presence of osteoclasts.
[l]ES sometimes used as an additional referent.
[m]Bone perimeter is an alternative referent; note that expression must be 2D, not 3D.
[n]Also called surface density.
[o]Note wide variety of meanings presently given to the term "active."
[p]Often called labeled surface or tetracycline surface (double, single, or both).
[q]Or calcification.
[r]Or mineralization.
[s]Note ambiguity between "trabecular" as a type of bone tissue and as a type of individual structural element.
[t]Must specify whether calculated according to parallel plate or rod model, or measured directly.
[u]Many other synonyms given in text.
[v]Equivalent to rate of bone turnover.

importance of stereologic theory for proper sampling, measurement and dimensional extrapolation.

Referents: An absolute area, perimeter, or number measurement is useful only as an index of the amount of tissue examined, for which acceptable minimum values should be specified; the term "absolute" is not used in any other sense. Of the four types of primary measurement only width (or thickness) can be interpreted without a referent, which will normally be some defined and measured area or perimeter in the section. Since several referents are possible for virtually all measurements, the chosen referent must always be specified consistently and explicitly; when this is done terms such as "ratio" and "relative" are redundant and should not be used. If only one referent is used, or if measurements with the same referent are grouped together, the referent may need to be mentioned only once, but it must be repeated each time if there is any possibility of confusion.

Abbreviations: These consist of the first letters in the same order as the words in the name, without superscripts or subscripts. Each symbol component has only one meaning, as specified in Table 1, and no latitude in the choice of abbreviations is allowed. Single capital letters are used for the most frequent terms, a capital letter and an additional lowercase letter for less frequent terms, and a single lowercase letter for terms that are in some sense related to time. Double letter abbreviations must be demarcated by periods; in the absence of periods each letter is to be construed as a separate abbreviation.

Standard format

The same format is used for all measurements.

Source — Measurement/Referent

The source is the type of structure or region within a sample on which the measurement was made and will most commonly be cortical bone tissue (Ct), cancellous bone tissue (Cn), endocortical surface (Ec), or total biopsy core (Tt), but many other sources are in occasional use (Table 2) or can be defined using the lexicon (Table 1). Circumstances in which the source can be omitted from the name are detailed in the body of the text. Current practice is inconsistent in this respect; even when measurements have only been made on cancellous bone tissue, the source is almost always mentioned for some measurements (e.g., trabecular bone volume) and frequently omitted for others (e.g., osteoid volume and surface). The need for and the rules pertaining to referents were given earlier. The most commonly used referents are tissue volume (TV), bone volume (BV), bone surface (BS), osteoid surface (OS), and bone interface (BI), but many other referents can be defined for particular purposes (Table 2). The principal referents are related by the surface to volume ratios BS/BV (S/V in stereologic terminology) and BS/TV (S_V in stereologic terminology).

The application of the new system to some of the more commonly used measurements is illustrated in Table 5 and compared with selected examples of current terminology. Note that the recommended units are based on two units for length (mcm and mm) and two units for time (day and year), and that percent is preferred for dimensionless ratios. It is conventional to distinguish between static and dynamic measurements, the former not requiring tetracycline labeling, but it is perhaps more important to distinguish between primary measurements (Table 3) and derived indices (Table 4). By primary measurement is meant not the absolute raw data, but the use of no more manipulation of the raw data than is needed to express them in terms of a referent, or to divide by a constant such as the time interval between labels. Derived indices require more complex arithmetical manipulation and usually rest on one or more assumptions that should always be made clear. Derived indices should not be reported without the primary measurements from which they are derived.

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